TIME-SEQUENCE STUDIES OF COMBINATION 5-FLUOROURACIL AND RADIOTHERAPY IN A TRANSPLANTED ADENOCARCINOMA

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TIME-SEQUENCE STUDIES OF COMBINATION 5-FLUOROURACIL AND RADIOTHERAPY IN A TRANSPLANTED ADENOCARCINOMA.

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DEDICATION :

To My Mother and Father

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INTRODUCTION

From the time of the first attempts at cancer chemotherapy with nitrogen mustards introduced at Yale by Gilman in 1942 (13) many classes of compounds have been developed. Among these are the alkylating agents and antimetabolites as recently reviewed by Boyland (5), the antibiotics and antibiotics as reviewed by Arbin (2), and the folic acid antagonists reviewed by Jacobs (25). Despite the multiplicity of agents, only one drug, methotrexate, has resulted in five year regressions in only one tumor, choriocarcinoma in women (5). All of these authors have further referred to the toxicity, potentially fatal, of most of the drugs at the doses that must be used for tumor regression. Recently interest has turned toward the use of the halogenated pyrimidines, summarized by Welch, Calabresi and Prusoff 1962 (39).

Within the last decade, the technical side of radiotherapy has rapidly progressed with the addition of greatly improved supervoltage x-ray machines generating 1 to 70 million electron volts and teletherapy with easily maintained cobalt 60 sources, plus the addition of isotope therapy (29). However, radiotherapy still is not a totally satisfying means of tumor therapy. Our attention has been drawn to the possibility of augmenting the effectiveness of radiation on the cell or sensitizing the cell to radiation by combination of x-ray with various chemical agents in the hope of increasing the therapeutic effect while holding toxic effects within acceptible levels. Furthermore, it has been our purpose to attempt to discover if an optimum time-sequence of administration exists in such combination regimens.

Bensley et al (4) have reviewed the field of combination therapy (1) dividing the various chemical agents used into categories: a) agents that increase susceptibility to x-ray either by effect of their own toxicity and secondary radiators or fixation. Among the sensitizing agents discussed are the alkylating agents, xanthines and the purine antimetabolite 6_2. various dyes, and synkavit are mentioned among the sensitizers. Spencer et al (35) used L-triiodothyronine in combination with 6500r x-ray on mouse breast tumors but noted no significant difference between combined therapy and x-ray alone. After an initial tissue edema, tumors did regress in size, but there was also an increase in the mortality from irradiation used in combination with the drug. Wolk has also been done using vasodilators (acetylcholine and Priazoline) with 2600r x-ray but only suggestive tissue if the expected oxygen effect have been shown (37). Attempts have been made by Baldin and Martel (15) to obtain therapeutic synergism (an effect greater than would be expected by adding the results of each therapy alone) by using two drugs together without x-ray. In most cases, toxicity as well as activity was reduced, but some 'enhanced effectiveness' was obtained with three serum factors given in advance of aminopterin. Leucutia (20) obtained marked and long palliative effects with colloidal lead orthophosphate and x-ray in some neoplasms and with nemolicosis and x-ray in multiple myeloma.

Kligerman (20) has evaluated some of the various chemicals that have been used in combination with x-ray and the evidence that exists for radiosensitization. Included are the original studies of the role iron or 5-iododeoxyuridine. Presently great interest is centered in the halogenated pyrimidine analogues. Corbaley (36) has demonstrated, that dose sufficient

[illegible] deficiency in the formation of deoxyribonucleic acid (DNA) and [illegible] acting as an antagonist to ribonucleic acid (RNA) synthesis. Initially orotic acid the precursor compound is converted to the pyrimidines cytosine, thymine and uracil. Heidelberger (21) has shown that 5-FU can take the place of uracil in metabolism without losing its fluoride ion which passes for hydrogen and blocks the position of normal methylation of the uracil. Thus 5-FU can go through normal anabolism to give a fraudulent DNA as well as in some way inhibiting its formation. Thymidinedeoxyribophosphate (TDPP) is known to be formed from Uridinedeoxyribonphosphate (UDPP) by a methylation reaction catalyzed by thymidylate synthetase in the presence of tetrahydrofolic acid. 5-FU is metabolized to form 5 fluoro 2 deoxyuridine 5 monophosphate which in turn has been discovered to be the substance that inhibits thymidylate synthetase activity. This inhibition seems to be the crux of the chemotherapeutic action of 5-FU. Combination therapy with 5-FU does not seem to cause any additive inhibition of thymidylate synthetase. Instead, Heidelberger has evidence that the presence of irradiation causes a block in the induced formation of thymidine kinase before this enzyme has a chance to rise as it would in normal conditions. This may play some role in sensitization but exactly how combination therapy exerts its effect is unknown. Heidelberger and his co-workers have thoroughly investigated the action of 5-FU illustrating the decreased inhibition of thymidylate synthetase in resistant tumors, the requirement of ATP for the action of 5-FU, and the failure of formate C^{14} to be incorporated in DNA thymine in the presence of 5 fluoro 2 deoxyuridine 5 monophosphate.

Experiments with 5-FU alone have demonstrated variable effects on a wide variety of neoplasms. Heidelberger (22) has obtained the following

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Tumor type	Major results
775 mammary adenocarcinoma	Slightly effective
Sarcoma 180	Carcinostatic
Sarcoma A-1	Slightly effective
E0771 Mammary adenocarcinoma	Inhibiting
Flexner-Jobling carcinoma	Initially effective
Walker 256 Carcinoma	Slightly effective
Ehrlich Ascites Carcinoma	Causes regression
L1210 Leukemia	Increased survival time
Yoshida Ascites sarcoma	Increased survival time
Novikoff hepatoma	Increased survival time

Vermund et al (27) investigated the use of combination therapy on transplanted adenocarcinomas in mice using 300r daily for 5 days amounting to a total of 5,100r of x-ray in 23 days and 35 mg/kg of 5-FU for 5 days then 17.5 mg/kg twice weekly for two weeks. This regimen of therapy gave an average survival time in Swiss albino mice with sarcoma of 73.9 and 67.2 days as against an average survival of 25.6 and 18.9 days in the control mice. Eight out of 19 mice showed complete regression of tumor with 5-FU and x-ray whereas x-ray alone merely inhibited tumor growth and caused no regression. 3 mice with an adenocarcinoma seemed to respond to combination 5-FU and x-ray therapy having an average survival of 72.8 and 71.2 days as compared with 42 and 53.8 days in the control groups. Only one of the tumors disappeared completely. These results are difficult to evaluate in terms of the effect on humans. The results in far advanced malignancy have, for the greater part, been inconclusive. Generally speaking, 5-FU given alone is

mice cells (Goldin, Venditti, Kline, Kellerman, Lomen and Shnider, 1971). The validity of comparing the results obtained with spontaneous and transplanted tumors in mice to human tumors is unknown. Also, the response of mouse tumors to combination therapy has been noted by Heidelberger (22) to vary, but, in general, he has noted an appreciable tumor inhibiting effect especially with 5-FU and Methyl CCNU in combination on ca cons 180 and 775 mammary adenocarcinoma where there is evidence of potentiation (22).

In humans, Gold and Gall (14), Winston et al (40) and Olson and Greene (31), have found that 5-FU alone has caused regression in 19.6% of all carcinomas treated with the longest remissions noted in cancers of the breast and uterus (50.8% experiencing some relief). Gall et als (17) have found that 5-FU is most effective in cancers of the breast, ovary, large intestine, rectum and in hepatomas.

The conventional doses of 5-FU in therapy are 15 mg/kg/day for 3 days by rapid IV injection (not to exceed 1 gm per day) and 7.5 mg/kg every other day until toxicity appears. Although possessing a definite anti-tumor effect, 5-FU has a very narrow therapeutic index (Olson et al (31) noting 18% fatal toxicity among their patients). The most common toxicities consist of stomatitis (40%, Pfeugeson & Humphrey in Olson et al (31); nausea, vomiting, or diarrhea (37%); leukopenia (70%), anemia and ulceration of the gastro-intestinal tract (11). Delayed effects include alopecia and increased pigmentation of the skin (16). A sudden agranulocytosis can occur after cessation of treatment (Olson et al) (31) and Winston et al (40) noted a high frequency of euphoria after using 5-FU. Recent work has been aimed at methods of alleviating this high toxicity while maintaining the anti-tumor effect. Some reduction in

MATERIAL AND METHODS

A mammary adenocarcinoma which arose spontaneously in our C3H colony and had been subcultured for many generations was transplanted into the left thigh of 215 mice. 60% of these mice were C3H/CCRL and the remainder C3H/JAX. All but 42 were males. In general, the mice were 4-6 months old at the start of the experiment.

A mouse carrying the tumor was sacrificed without anesthesia, the tumor being removed and minced under saline. Fifty C3H mice were then innoculated with minced tumor by a size 13 trocar in the left thigh. About 10-12 days after transplantation, 42 of the 50 mice whose tumors were subcutaneously measurable were selected. Only tumors of fairly uniform size (7-9 mm average diameter) were used in order to reduce bias. These 42 mice were then assigned randomly to 7 groups of about 6 animals each.

X-ray treatment was carried out in 1/4" thick boxes with the animal's left leg held out by a string and placed directly under the 1.5 cm. cone. No anesthesia was used.

With day 0 as the day of initiation of treatment, all tumors were measured on the following fixed days: #1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 19, 26, 33, 40. Tumor volume was calculated by multiplying the three dimensions together, as measured by a standard caliper, and multiplying this figure by .524. Most of the treated mice and all of the untreated ones died of metastasis within this 40 day observation period. For the duration of the period of observation, the various groups were assigned randomly to several cages to avoid the cage effect as described by Bayonet (3).

thereby, discussion of the final results is made within the parameters of the one test conducted.

Therapy, as mentioned, consisted of both x-irradiation and 5-fluorouracil. One intraperitoneal injection of 125 mg/kg of 5-fluorouracil (which is determined by preliminary dose-response studies but on LD_{10} to LD_{50} treatment) was used throughout. This was an effective dose with the highest acceptable mortality rate. The x-ray dose chosen (based on prior experiments) was one local treatment of 5000r, an ED_0 for long-term regression of tumor cells. These doses were selected to insure that any additive or enhancement effect would not be masked. The machine was set at 250Kv, 15 ma, 0.15 mm Cu filter. The cone length was 3 cm and the distance from the cone to the target was negligible. Victoreen readings of the 1000r chamber timed for one minute of machine output averaged about 900r/min. corrected.

The treatment groups received the following regimens:

Group I - received local x-ray dose of 5000r.

Group II - received an intra-peritoneal injection of 5-FU; dose - 125 mg/kg (LD_{10}).

Group III - received the injection of 5-FU and 15 minutes later received 5000r x-ray.

Group IV - received the injection of 5-FU and 3 hours later received 5000r x-ray.

Group V - received the injection of 5-FU and 24 hours later received 5000r x-ray.

Group VI - received 5000r x-ray and 24 hours later received the injection of 5-FU.

Group VII - served as the control and received no treatment.

RESULTS

Emphasis is placed on the progression of tumor volume following the initial period of therapy. Other parameters followed included tumor take and survival times.

The variability of response of the individual mice in a group (differences within each group were greater than the differences between groups. The mice which died early were considered deaths by toxicity.

To analyze, averages were made of all changes in each of the groups for each day and the corresponding groups logarithmically plotted together for comparison. In figure 1, the tumor sizes of the groups receiving x-rays alone (Group I) are plotted, the number of surviving mice being indicated by a number above each point on the graph. It should be noted that the point about Day 5 was the only run employing female C_3H mice. Sex, however, did not appear to be a factor affecting the results. The groups with 5-Bromodeoxyuridine (Groups II, III, IV, V) (figure 2), with the exception of one mouse, show no response in any way to the drug alone. Giving the drug 15 minutes prior to x-ray therapy (Group III) (See figure 3) seems to have caused an inhibition of tumor growth after 5-6 days lasting until about the fifteenth day. At this point, growth of the tumor inevitably resumed. The results of the group receiving 5-BU three hours prior to x-rays (Group II) (See figure 4) are similar to Group III. There is inhibition in tumor growth and even some regression of tumor size followed by regrowth of the tumor in greater than 75% of the remaining mice. The groups receiving 5-BU 24 hours prior to x-rays (Group V) (See figure 5 resemble Group IV with the exception of the existence of a few greater extremes

in tumor size. In the group receiving x-rays 2 hours prior to each dose of [illegible] (See figure 6), there are results quite variable but not significantly different from those of the other combined treatment groups. The tumors of the controls (Group VII) (figure 7) grow quite rapidly as is expected. One mouse in Run 3 had an unusually long survival, dying only after a very large tumor had grown.

For further consolidation of the results, all of the tumor volumes in each treatment group, were averaged together and plotted as one curve (figure 8). The general trend for each group is clearly illustrated, but one must be mindful that the fewer the surviving mice, the less representative are the average tumor sizes. Standard errors of the mean were calculated at each day of observation and are represented on separate graphs for each treatment group (figures 9-15). The standard errors, based only on the surviving animals, became unreliable after day 19 because of attenuation of the colony due to tumor deaths. The standard error for each group at day 19 is recorded on the composite graph (figure 8). The number of survivors in each treatment group at fixed days after treatment is shown in Table I.

Up to the 19th day, there is no statistically significant difference between the group receiving x-ray alone (Group I) and any of the combination therapy groups (Groups III-VI). After the 19th day, a general increase in average tumor volume is noted in all of the combination therapy groups, whereas a slight decrease is seen in the group with x-ray alone (Group I). This late difference cannot be subjected to statistical analysis due to the small number of survivors. Likewise, there is little difference between the control group (Group VII) and the group receiving chemotherapy alone (Group II). The last point on the latter curve represents only one mouse whose tumor had

TABLE I

SURVIVORS

Day	0	5	9	15	19	26	33	40
Group I X-ray - 5000 r	30	30	30	30	28	24	16	14
Group II 5-FU	31	31	25	24	21	12	3	1
Group III 5-FU 15 Min. Prior to 5000 r	31	31	24	24	24	22	15	9
Group IV 5-FU 3 Hrs. Prior to 5000 r	32	32	27	26	25	19	15	9
Group V 5-FU 24 Hrs. Prior to 5000 r	31	31	25	24	24	23	15	8
Group VI 5-FU 24 Hrs. After 5000 r	30	28	23	21	19	16	10	9
Group VII Control	29	29	28	21	13	2	2	1

continuously increased in size after chemotherapy alone until the 19th day and then began to regress until the death of the mouse. Therefore, this point should be disregarded. This is the only incidence of tumor regression in either of these 2 groups (II or VII). Both of these groups, however, differ significantly from all those receiving x-ray. It is noteworthy that in no group was the final average tumor volume smaller than the starting average tumor volumes, although the group with x-ray alone (Group I) approached this. (Figures 9-15 show the average tumor sizes of each group separately with the standard errors included).

Since we were working with transplanted tumors, the most meaningful data is represented by the regression and growth rates. (Host factors influence tumor 'cures'). However, Tables 2a and 2b are included to show detailed data on permanent regression of the primary tumor and complete regression followed by recurrence. The causes of death following treatment are listed in Tables 3a and 3b. Most mice died of metastases from the implanted tumor including those that obtained temporary 'cures' at the original tumor-site (tumor no longer palpable or observable). The mice listed as 'permanent regression' had no evidence of original tumor or metastasis when sacrificed 4-5 months after the 40 day observation period. The x-ray dose (5000r) given to Group I gave a temporary local cure of 40% (FD_{40} for regression of local tumor followed by recurrence). This same degree of effectiveness was not noted in any of the other groups receiving 5-FU in addition to the same x-ray dose. In fact, the percentage of temporary complete regression of local tumor ranged from a low of only 25% in one of the combination groups to a high of 36% in one of the other combination groups, the difference between groups not being significant. An FD_8 for permanent regression of local tumor was obtained

TABLE 2A

PERMANENT REGRESSIONS

Runs	1	2	3	4	5	Total
I 5000r X-ray	0/6	0/6	0/6	1/6	1/6	2/30
II 5-FU	0/7	0/6	0/6	0/6	0/6	0/31
III 5-FU 15 Min. Prior to 5000r	2/6	0/6	0/7	0/6	1/6	3/31
IV 5-FU 3 Hrs. Prior to 5000r	1/7	0/7	0/7	0/6	1/6	2/33
V 5-FU 24 Hrs. Prior to 5000r	1/6	0/6	0/7	1/6	0/6	2/31
VI 5-FU 24 Hrs. After 5000r	0/6	0/6	1/6	1/6	2/6	4/30
VII Control	0/3	0/6	0/6	0/6	0/6	0/29

TABLE 3B

COMPLETE REGRESSION FOLLOWED BY RECURRENCE

Runs	1	2	3	4	5	Total
I 5000r X-ray	1/6	2/6	4/6	4/6	1/6	12/30
II 5-FU	0/7	0/6	0/6	0/6	0/6	0/31
III 5-FU 15 Min. Prior to 5000r	2/6	0/6	5/7	0/6	1/6	8/31
IV 5-FU 3 Hrs. Prior to 5000r	2/7	0/7	4/7	2/6	2/6	10/33
V 5-FU 24 Hrs. Prior to 5000r	0/6	0/6	5/7	3/6	1/6	9/31
VI 5-FU 24 Hrs. After 5000r	2/6	0/6	4/6	1/6	1/6	8/30
VII Control	0/5	0/6	0/6	0/6	0/6	0/29

TABLE 3A
CAUSE OF DEATH

	Original Tumor						Metastases					
Run	1	2	3	4	5	Total	1	2	3	4	5	Total
I 5000r X-ray	0/6	0/6	0/6	0/6	0/6	0/30	5/6	6/6	6/6	5/6	5/6	27/30
II 5-FU	0/7	0/6	2/6	0/6	0/6	2/31	5/7	6/6	3/6	3/6	5/6	22/31
III 5-FU 15 Min. Prior to 5000r	0/6	0/6	0/7	0/6	0/6	0/31	3/6	5/6	7/7	2/6	4/6	21/31
IV 5-FU 3 Hrs. Prior to 5000r	0/7	0/7	0/7	0/6	0/6	0/33	4/7	5/7	5/7	3/6	5/6	22/33
V 5-FU 24 Hrs. Prior to 5000r	0/6	0/6	0/7	0/6	0/6	0/31	2/6	5/6	7/7	4/6	4/6	22/31
VI 5-FU 24 Hrs. After 5000r	0/6	0/6	0/6	0/6	0/6	0/30	3/6	5/6	5/6	2/6	2/6	17/30
VII Control	2/5	0/6	0/6	0/6	0/6	2/29	3/5	6/6	6/6	6/6	6/6	27/29

TABLE 3B

CAUSE OF DEATH

	Drug Toxicity						Other (Pneumonia, Hepatitis, Other Infections)					
Run	1	2	3	4	5	Total	1	2	3	4	5	Total
I 5000r X-ray	0/6	0/6	0/6	0/6	0/6	0/30	1/6	0/6	0/6	0/6	0/6	1/30
II 5-FU	2/7	0/6	1/6	2/6	1/6	6/31	0/7	0/6	0/6	1/6	0/6	1/31
III 5-FU 15 Min. Prior to 5000r	1/6	1/6	0/7	4/6	1/6	7/31	1/6	0/6	0/7	0/6	0/6	1/31
IV 5-FU 3 Hrs. Prior to 5000r	1/7	2/7	2/7	2/6	0/6	7/33	1/7	0/7	0/7	1/6	1/6	3/33
V 5-FU 24 Hrs. Prior to 5000r	3/6	1/6	0/7	1/6	2/6	7/31	0/6	0/6	0/7	0/6	0/6	0/31
VI 5-FU 24 Hrs. After 5000r	3/6	1/6	0/6	2/6	1/6	7/30	0/6	0/6	0/6	1/6	1/6	2/30
VII Control	0/5	0/6	0/6	0/6	0/6	0/29	0/5	0/6	0/6	0/6	0/6	0/29

with 5-FU. That is, apparent cures occurred in [illegible] of the mice. No cures occurred in the mice treated with drug alone. The rest of the mice, i.e. those that were not cured or did not die of metastases (the large majority of which were in the combined therapy groups) died [illegible] in the course of the experiment from a side reaction of therapy. The dose of 5-FU given by itself was a LD_{19} under experimental conditions as indicated by the toxicity found in Group II (5-FU alone). The other groups receiving 5-FU (the combined therapy groups) had a slightly higher incidence of toxicity. In fact, there was an average of 23-30% fatal toxicities with combined therapy as against no such cases with x-ray alone. The miscellaneous causes of death were few and included events such as injection damage and infections that were present in the colony at the time.

Discussion

The above results revealed that not only did the timing of administration of therapy fail to play an important role, but also no combination regimen seemed to give greater antineoplastic effect than was possible with x-ray therapy alone. The lack of potentiation of radiation by 5-FU in these experiments correlate with the clinical report of von Essen and co-workers (38). This study, using x-ray alone, 5-FU alone, and combined therapy on multiple metastatic tumors within individual patients, failed to demonstrate a significant alteration of response of tumor to radiation by the addition of 5-FU.

There was a wide range in degree of effectiveness among the mice responding to therapy. Some mice did not respond at all to a therapy schedule that in other mice would cause a disappearance of the original tumor. Treatment in some was sufficient only to cause transitory inhibition in tumor growth followed by resumption of the previous growth rate (Fig.); in others, regression in size or even complete disappearance was followed by regrowth. In each case, it appeared as though the administered therapy regimen was only partially carcinolytic, the remaining viable cells surviving for the following regrowth. In some cases, these remaining tumor cells did not apparently have an opportunity to regrow since fatal metastases had already taken place.

There is the possibility that the administration of 5-FU even 24 hours prior to x-ray was inadequate time for full sensitization. Yet Bagshaw (3) has reported obtaining enhanced x-ray effects on HeLa cells when 5-FU was added only 4 hours prior to x-ray. Shpakoff (36), on the other hand, has noted that the sensitizing effects of purine and pyrimidine antimetabolites

SUMMARY

The effect of combination 5-FU and radiotherapy was tested by measuring the size of a transplanted adenocarcinoma in C_3H mice at certain fixed days after various sequences of drug and irradiation. Isotoxically effective doses of 5-FU (125 mg/kg by single I.P. injection, an LD_{10}) and x-ray (5000r locally, an TD_0 for permanent regression of local tumor) were used. The seven treatment groups were: (I) X-ray alone; (II) drug alone; (III) drug 15 mins. prior to x-ray; (IV) drug 2 hours prior to x-ray; (V) drug 24 hours prior to x-ray; (VI) x-ray 24 hours prior to drug; (VII) no treatment. The total study was divided into five small and identical experiments, with mice being randomly assigned to cages after thawing, so as to avoid chance events and spurious findings biasing the total result.

No significant difference was noted between those receiving x-ray alone and any other combination of x-ray and drug. Furthermore, there was no slight difference in growth rate between the controls and the mice receiving drug alone. Greater variation in response was noted within each group than between the groups.

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APPENDIX

GROUP I - X-RAY ALONE 5000R - AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

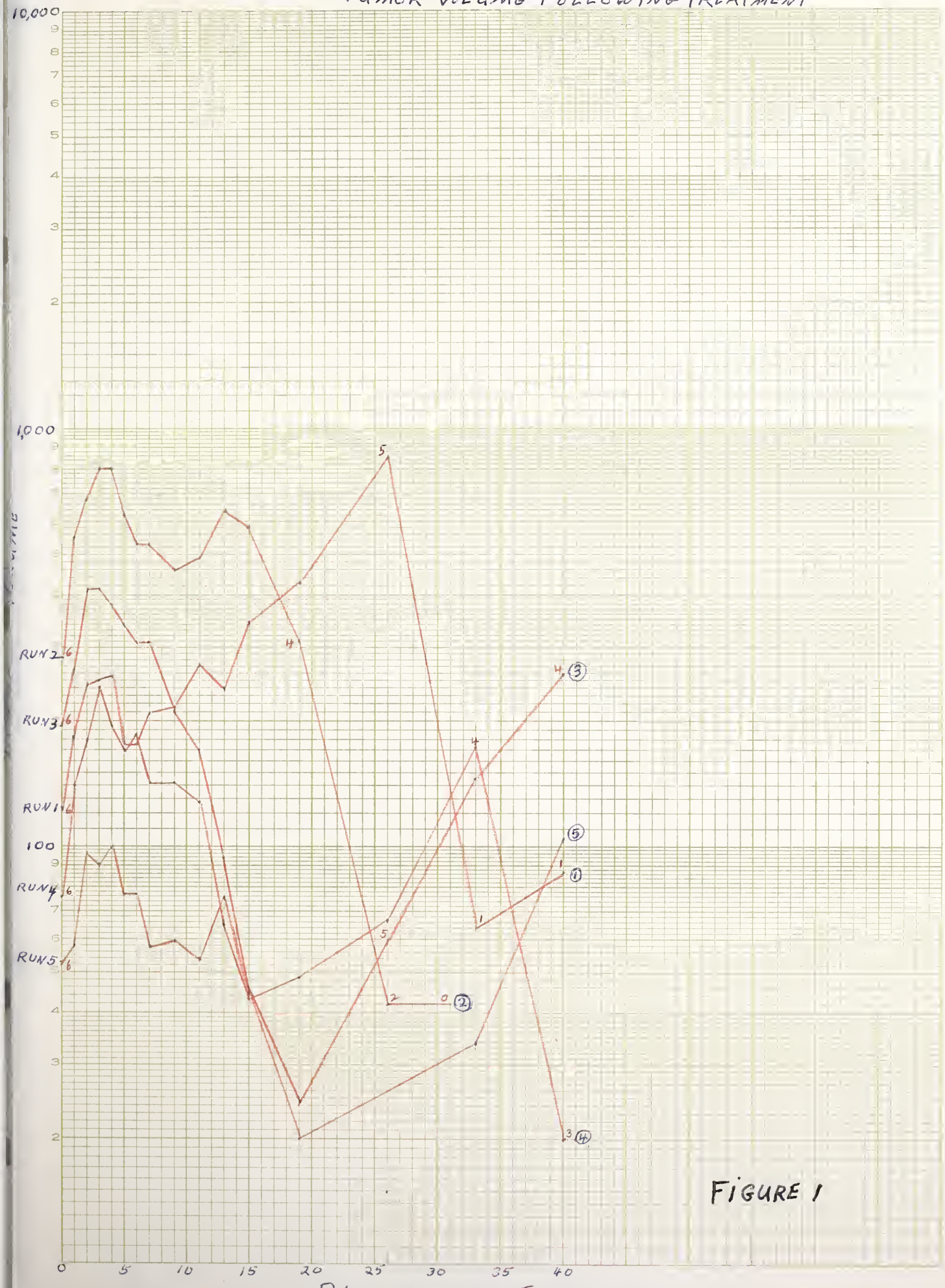
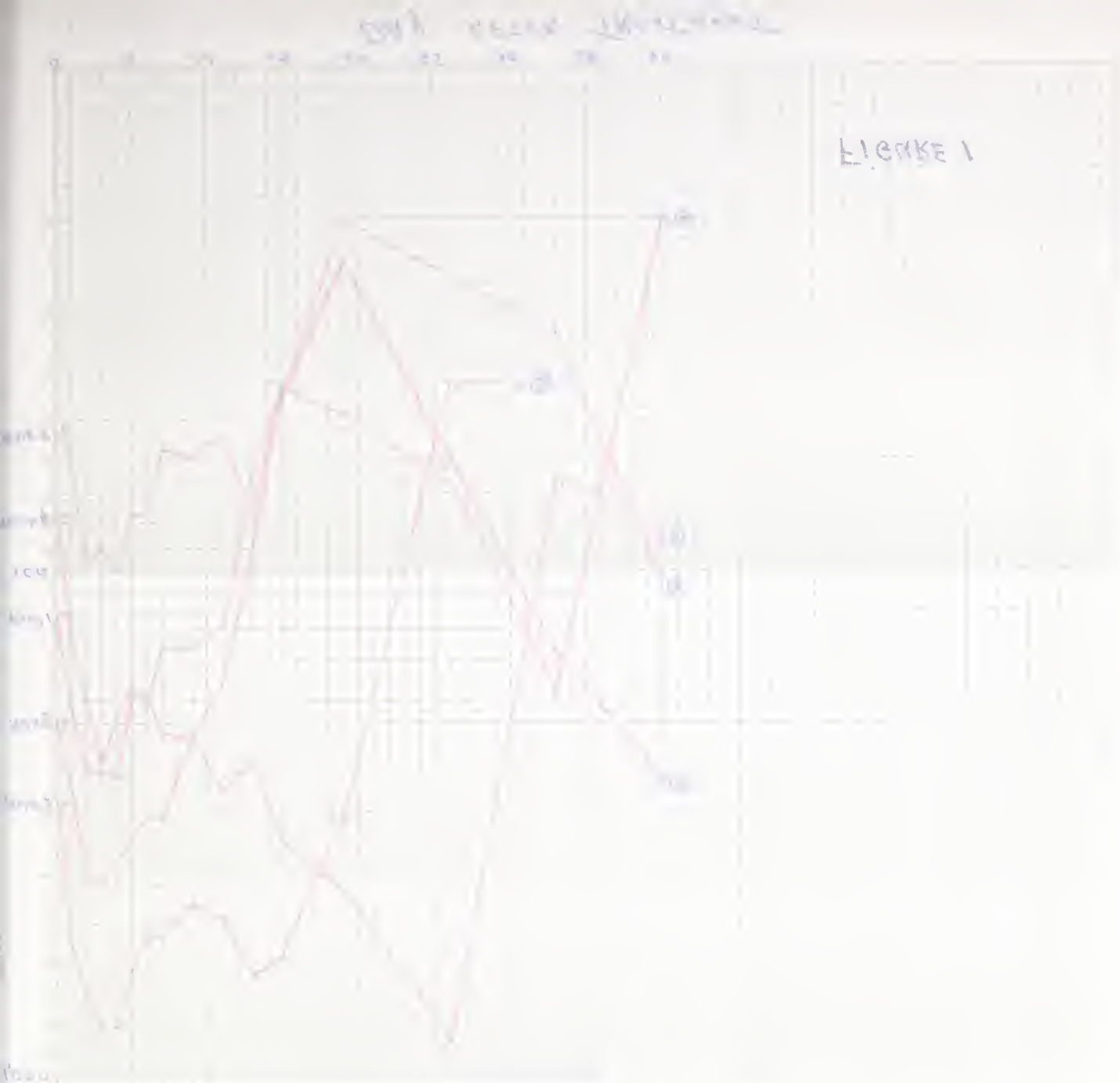


FIGURE 1



GROUP II - 5FU ALONE (125mg/KG = ⑤ LD10) AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

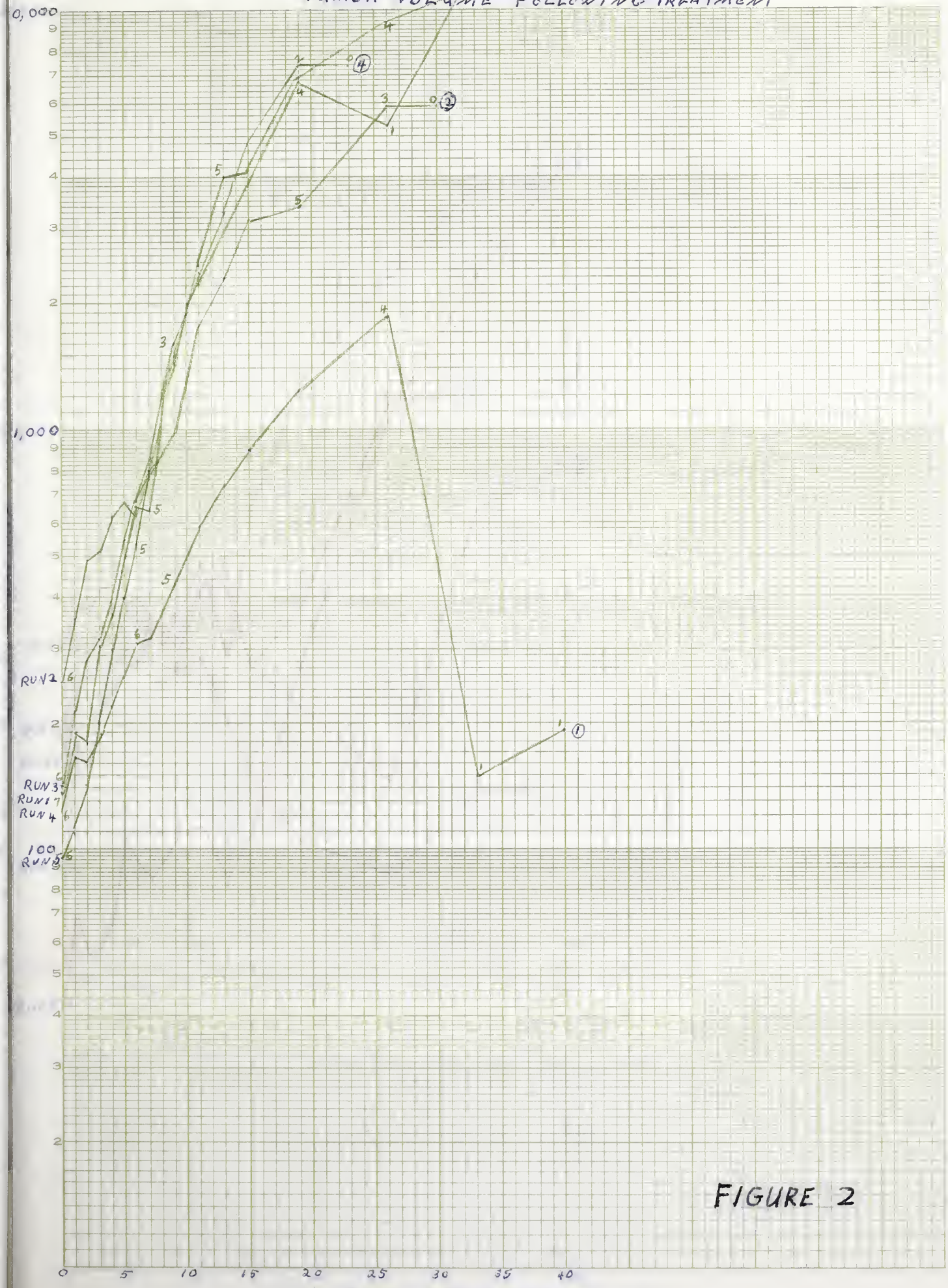


FIGURE 2

FIGURE 2

GROUP III - 5FU - 15 MIN. BEFORE X-RAY - AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

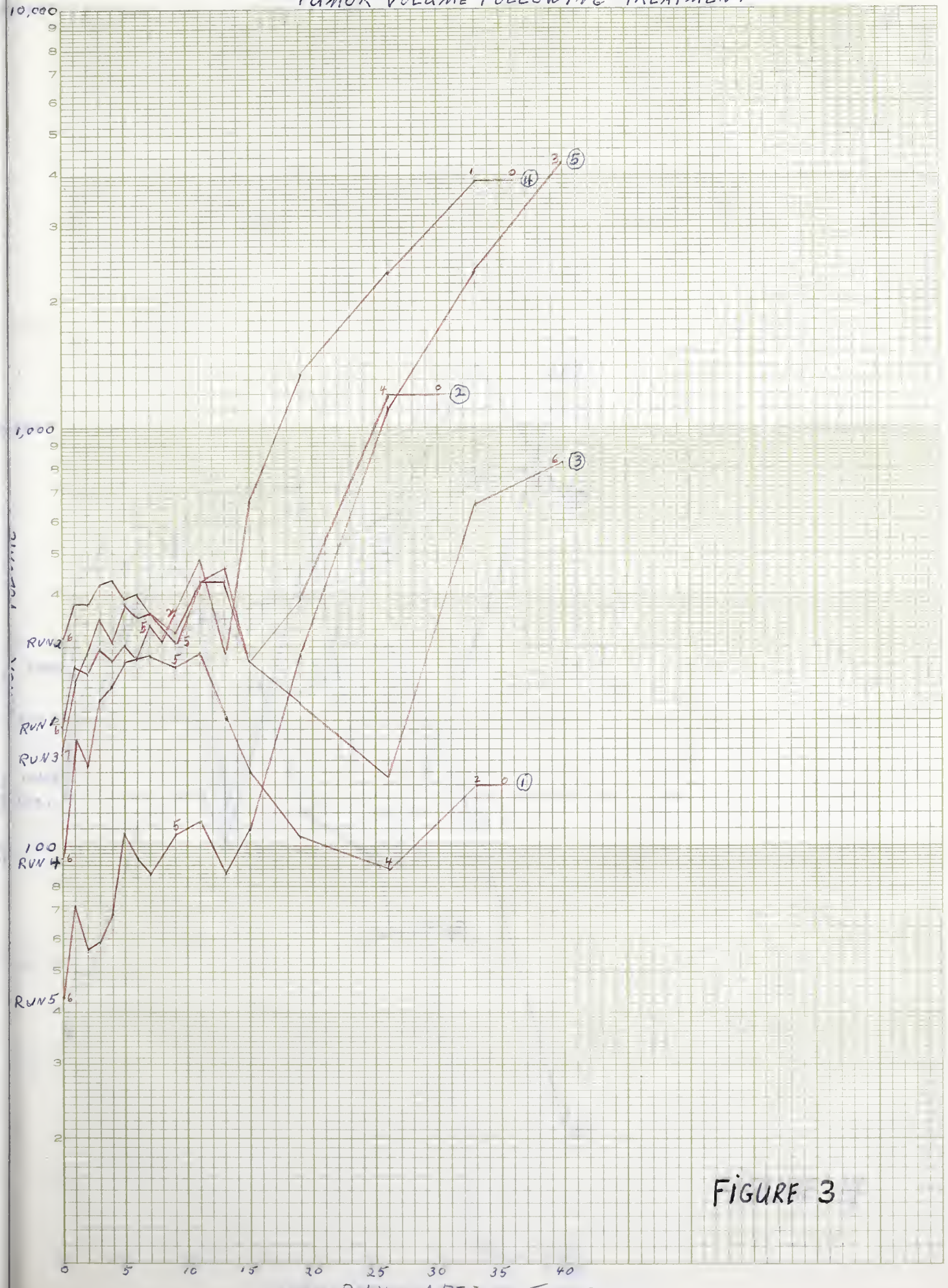


FIGURE 3

GROUP IV - 5FU - 3 HRS. BEFORE X-RAY - AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

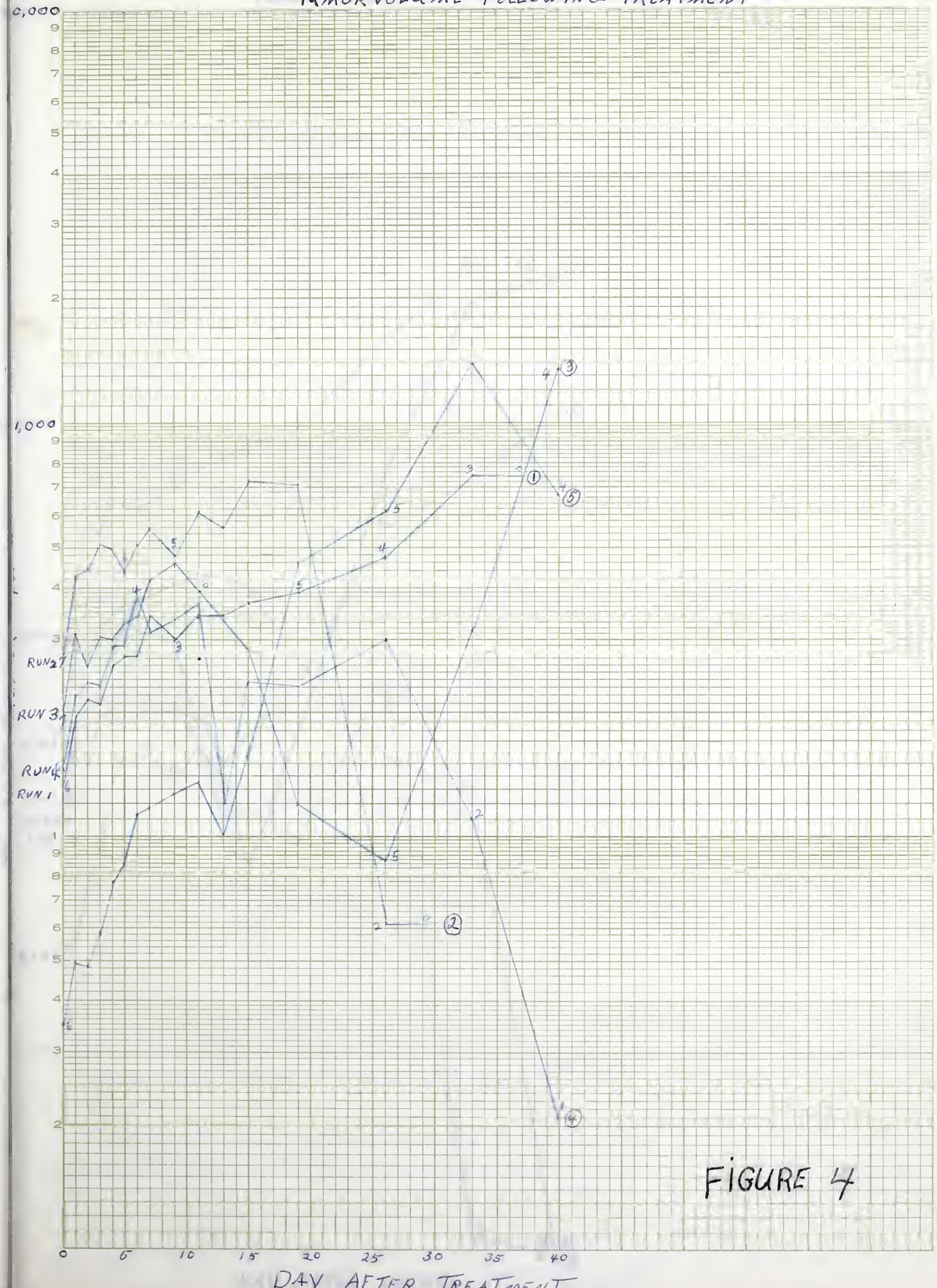


FIGURE 4

FIGURE 4

GROUP V- 5 FU - 24 HRS. BEFORE X-RAY - AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

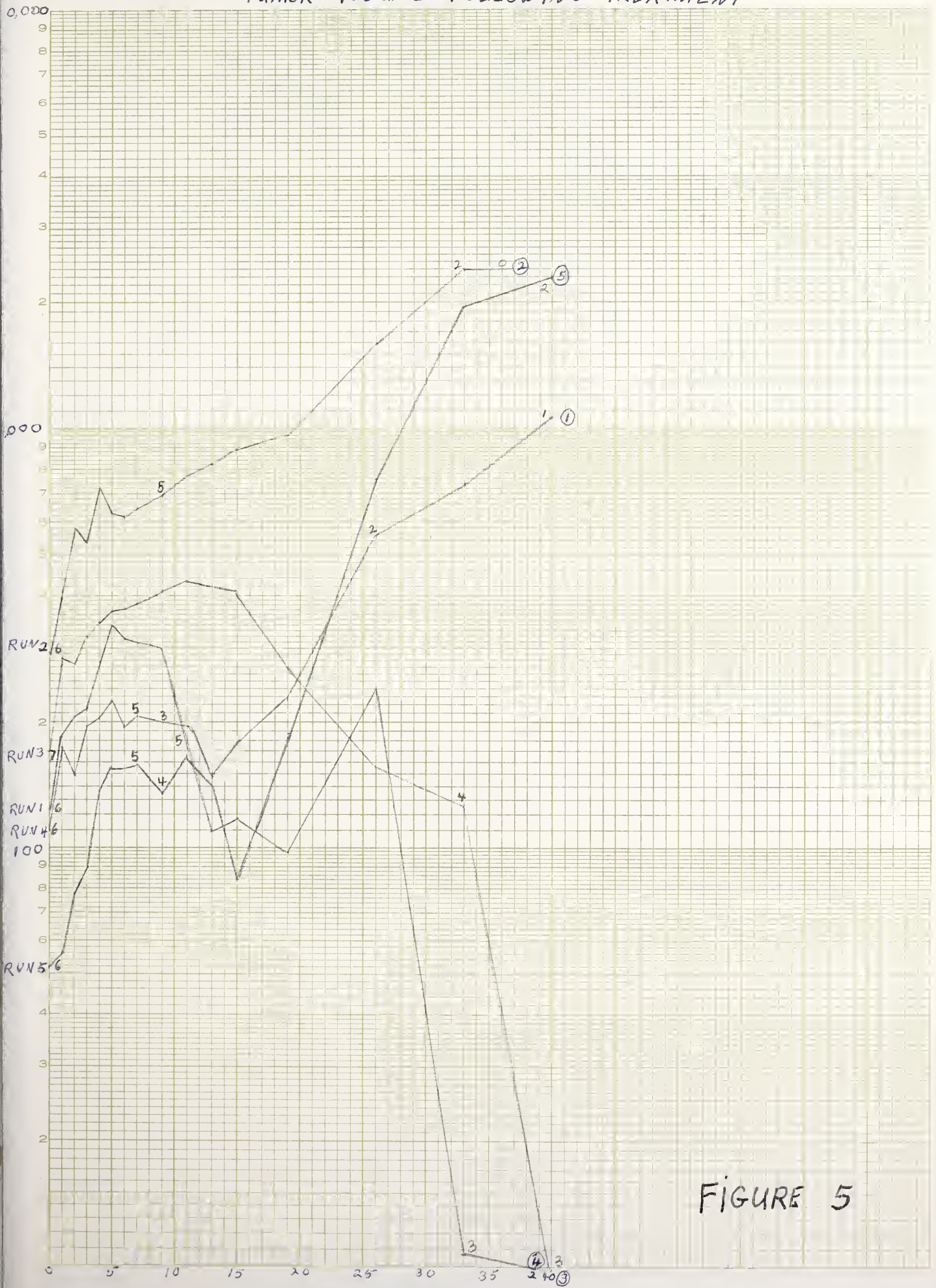


FIGURE 5

FIGURE 5

GROUP VI - X-RAY - 24 HRS. BEFORE 5FU - AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

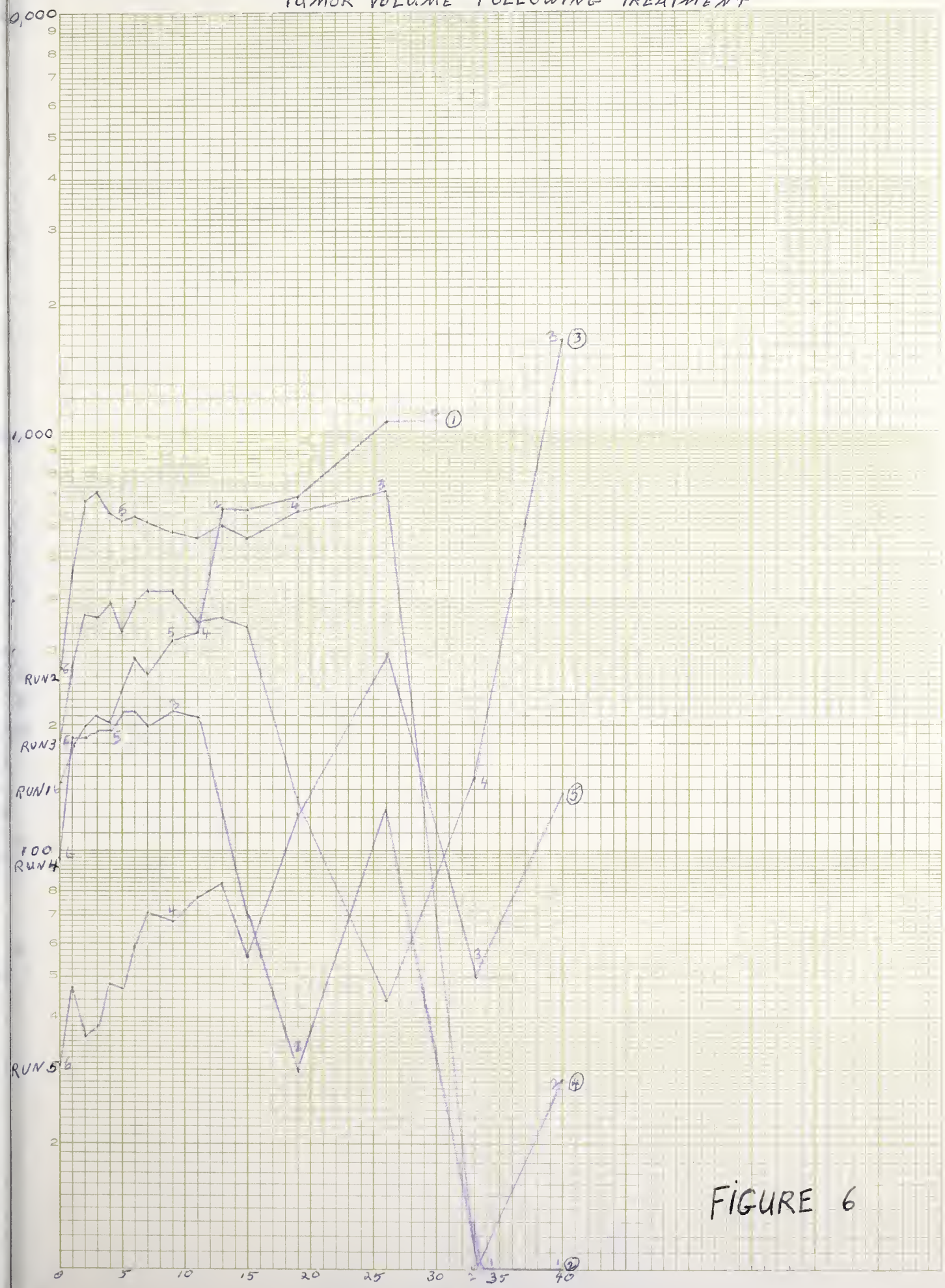


FIGURE 6

FIGURE 6

GROUP VII - CONTROL - ③ AVERAGE OF EACH RUN

TUMOR VOLUME FOLLOWING TREATMENT

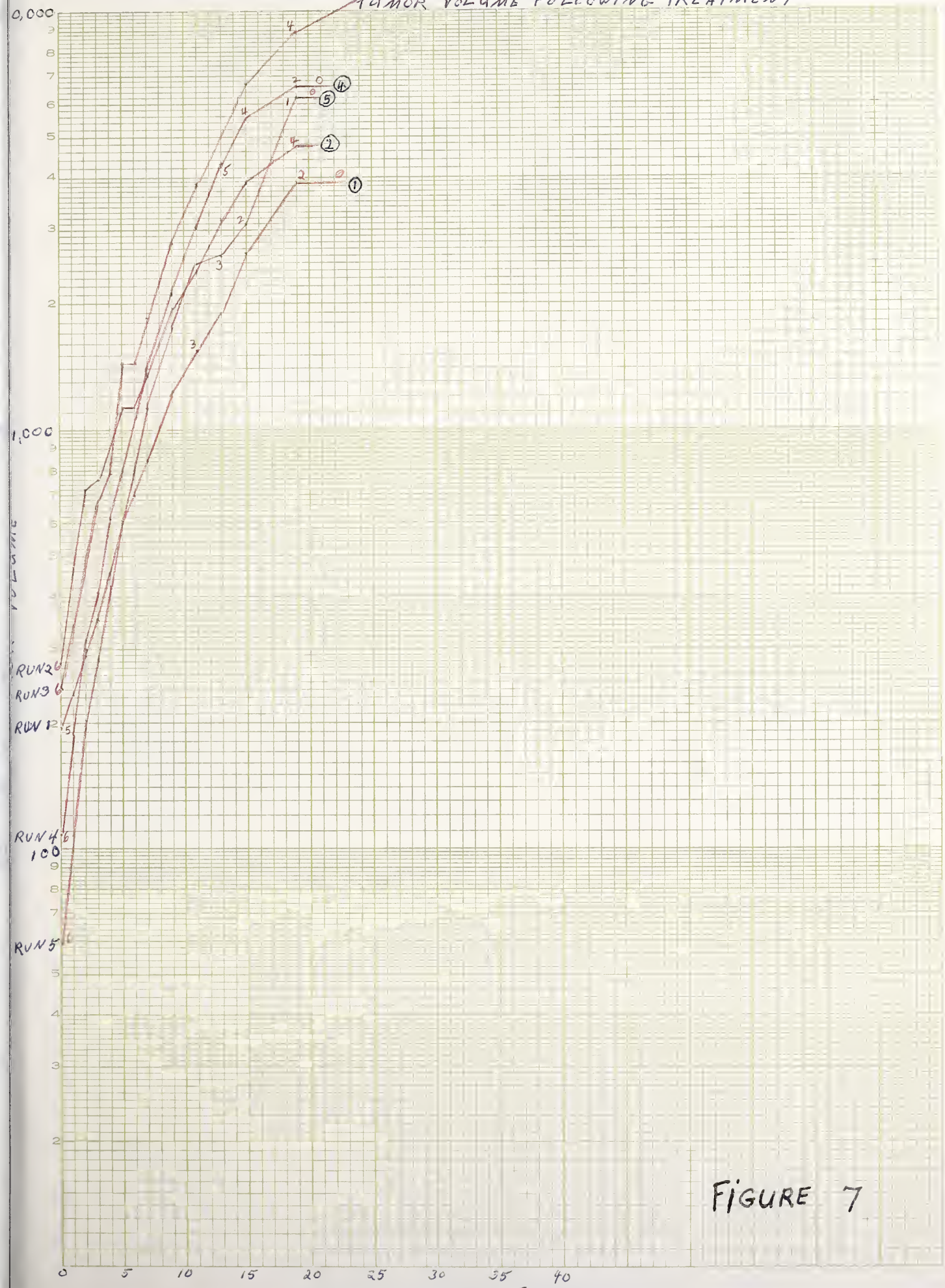


FIGURE 7


FIGURE 3

FIGURE 8 AVERAGE VOLUMES OF EACH GROUP

TUMOR VOLUME FOLLOWING TREATMENT

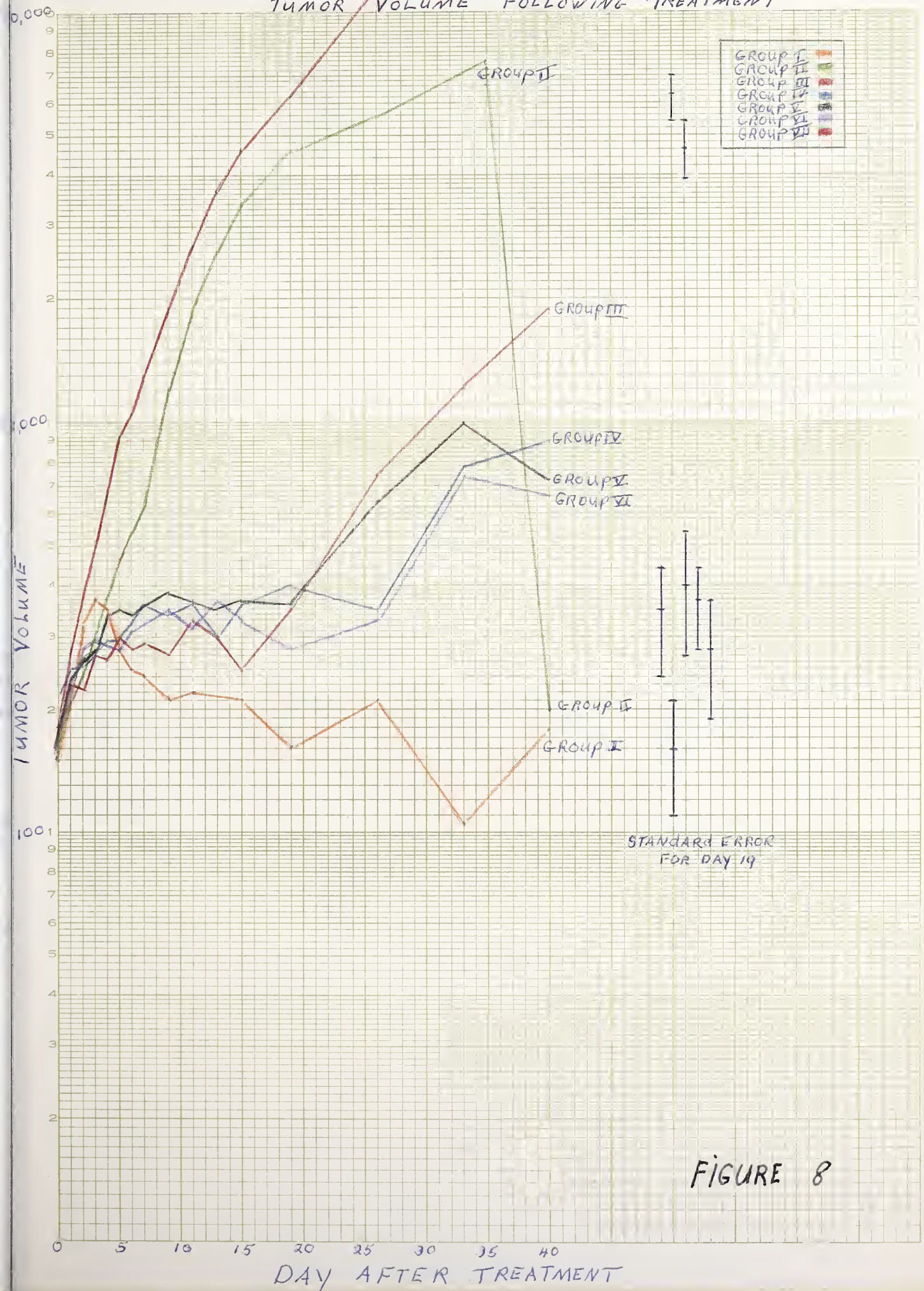


FIGURE 8

FIGURE 9 - GROUP I - X-RAY ALONE - 5000R

FIGURE 9

FIGURE 9

FIGURE 10 - GROUP II - 5FU ALONE (125 mg/kg = LD19)

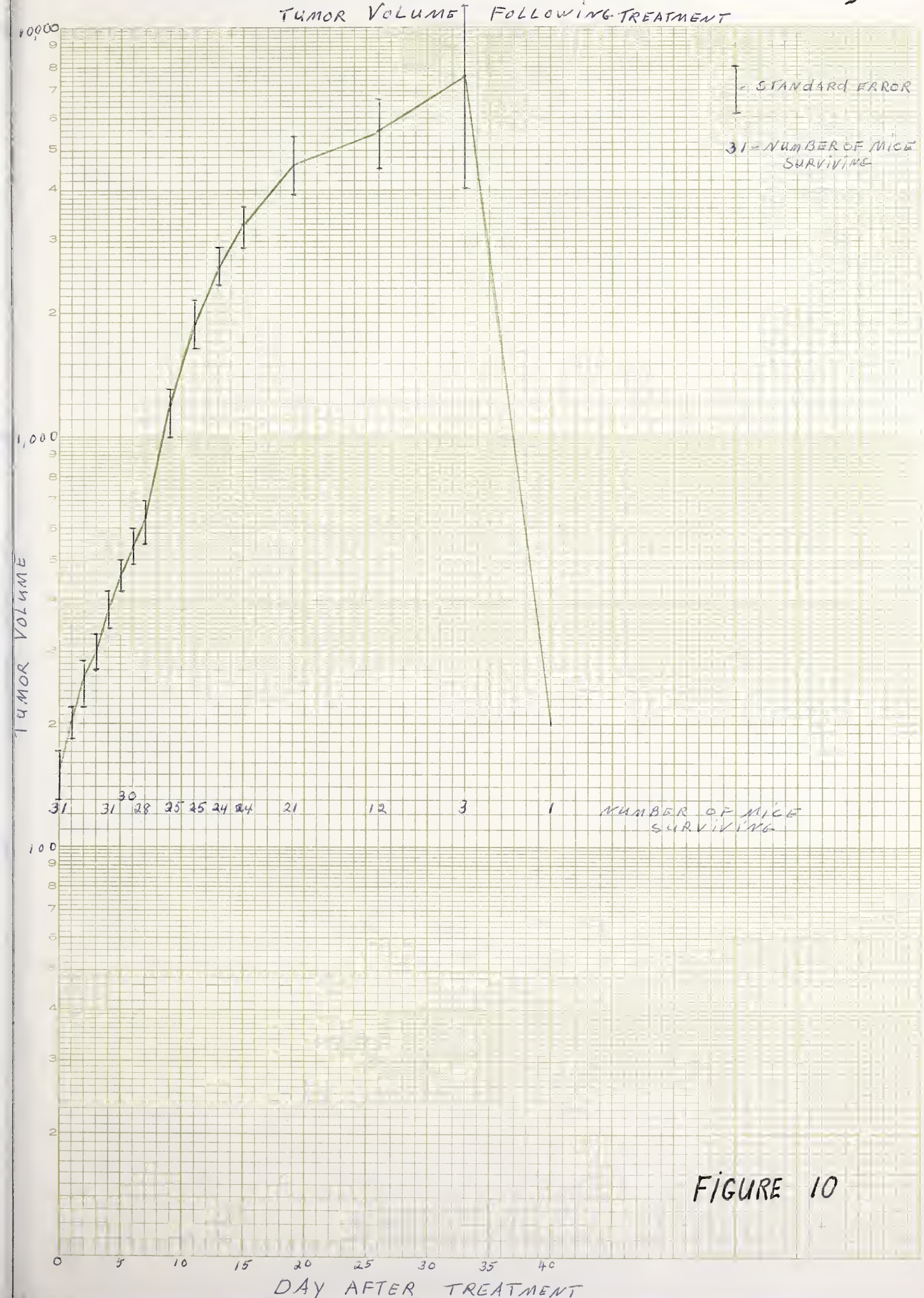


FIGURE 10

FIGURE II - GROUP III - 5FU 15 MIN BEFORE X-RAY

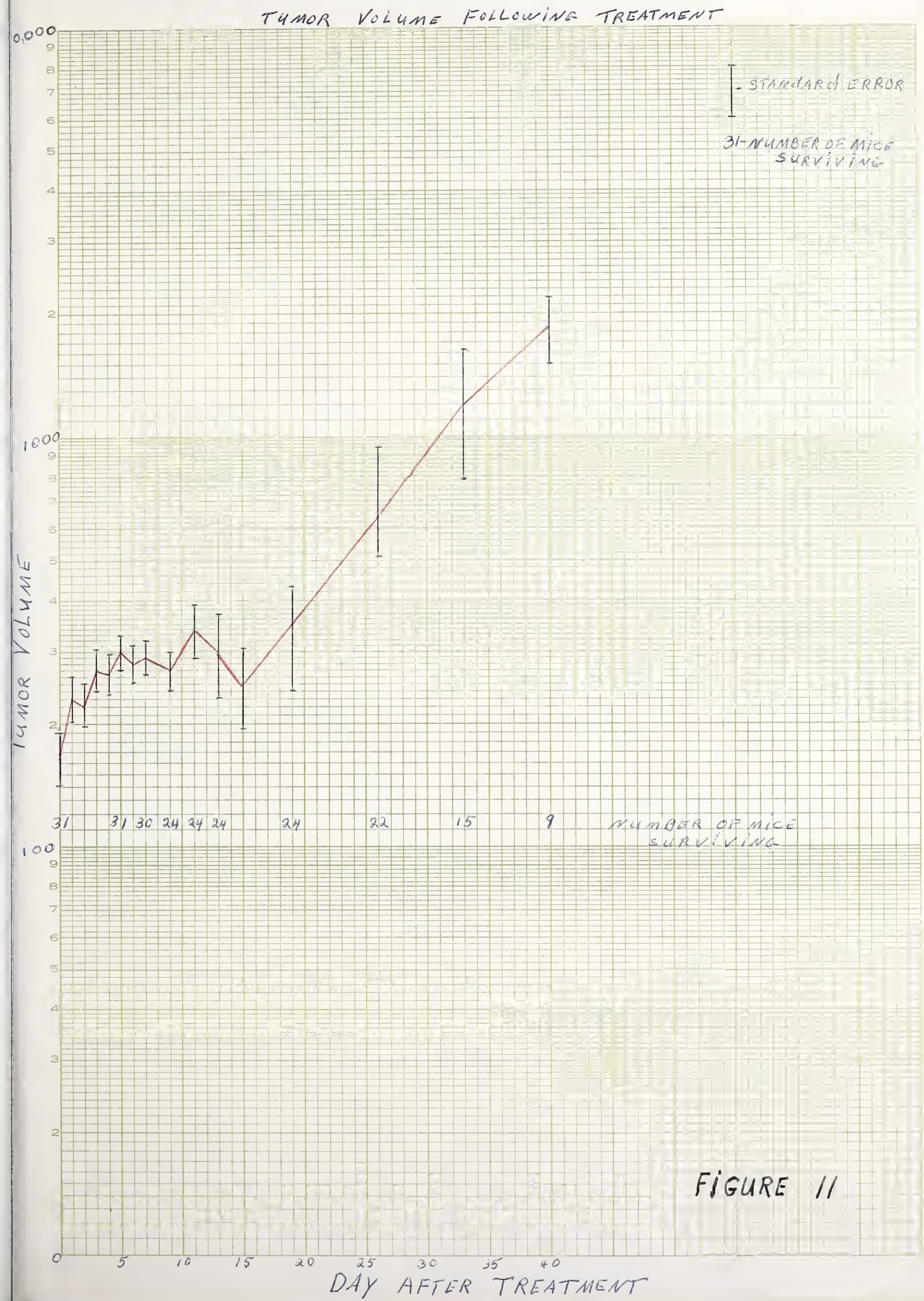


FIGURE II

FIGURE 11

FIGURE 12 - GROUP IV 5FU 3HRS. BEFORE X-RAY

TUMOR VOLUME FOLLOWING TREATMENT

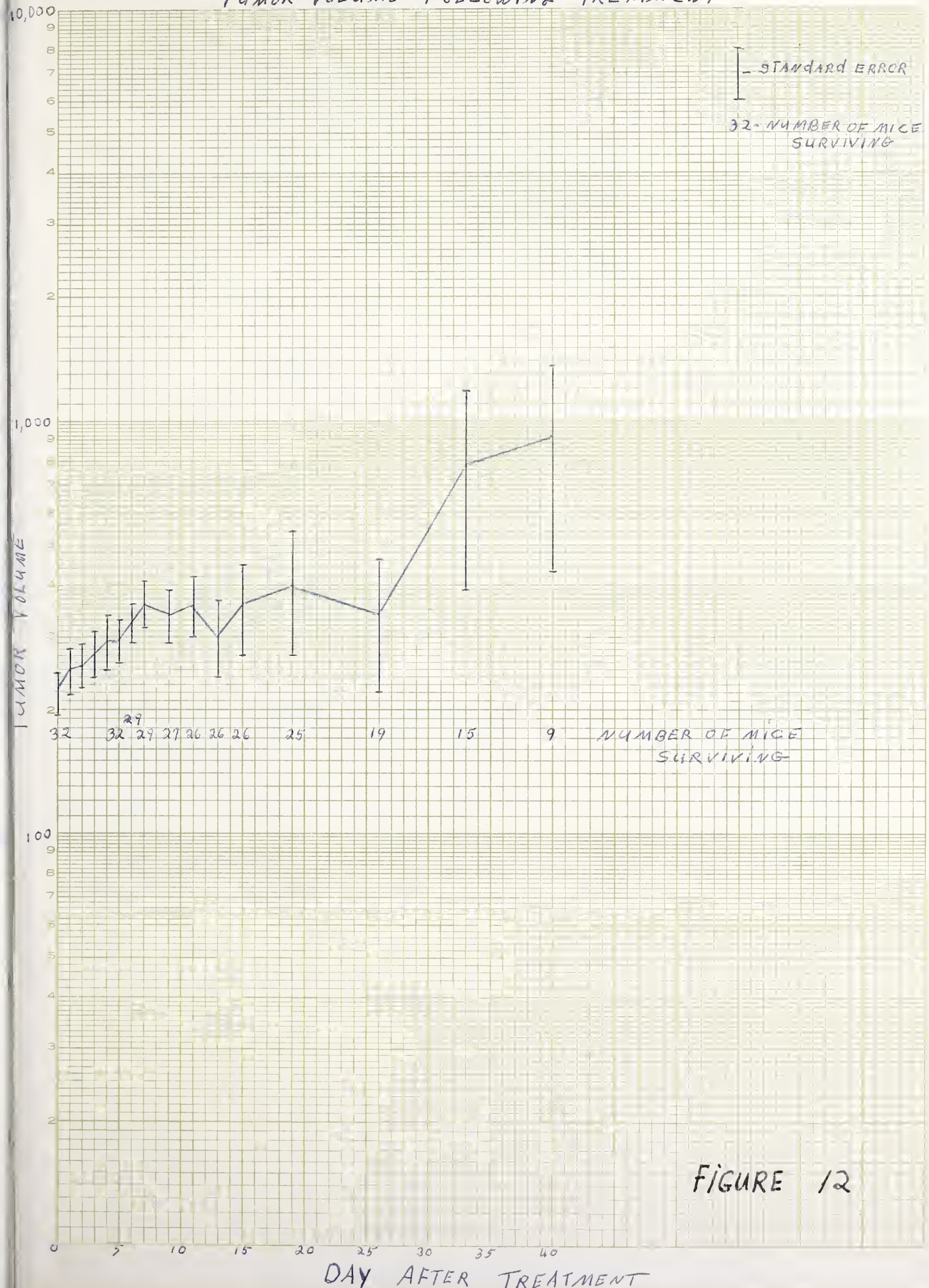


FIGURE 12

FIGURE 13 - GROUP V 5FU 24 HRS. BEFORE X-RAY

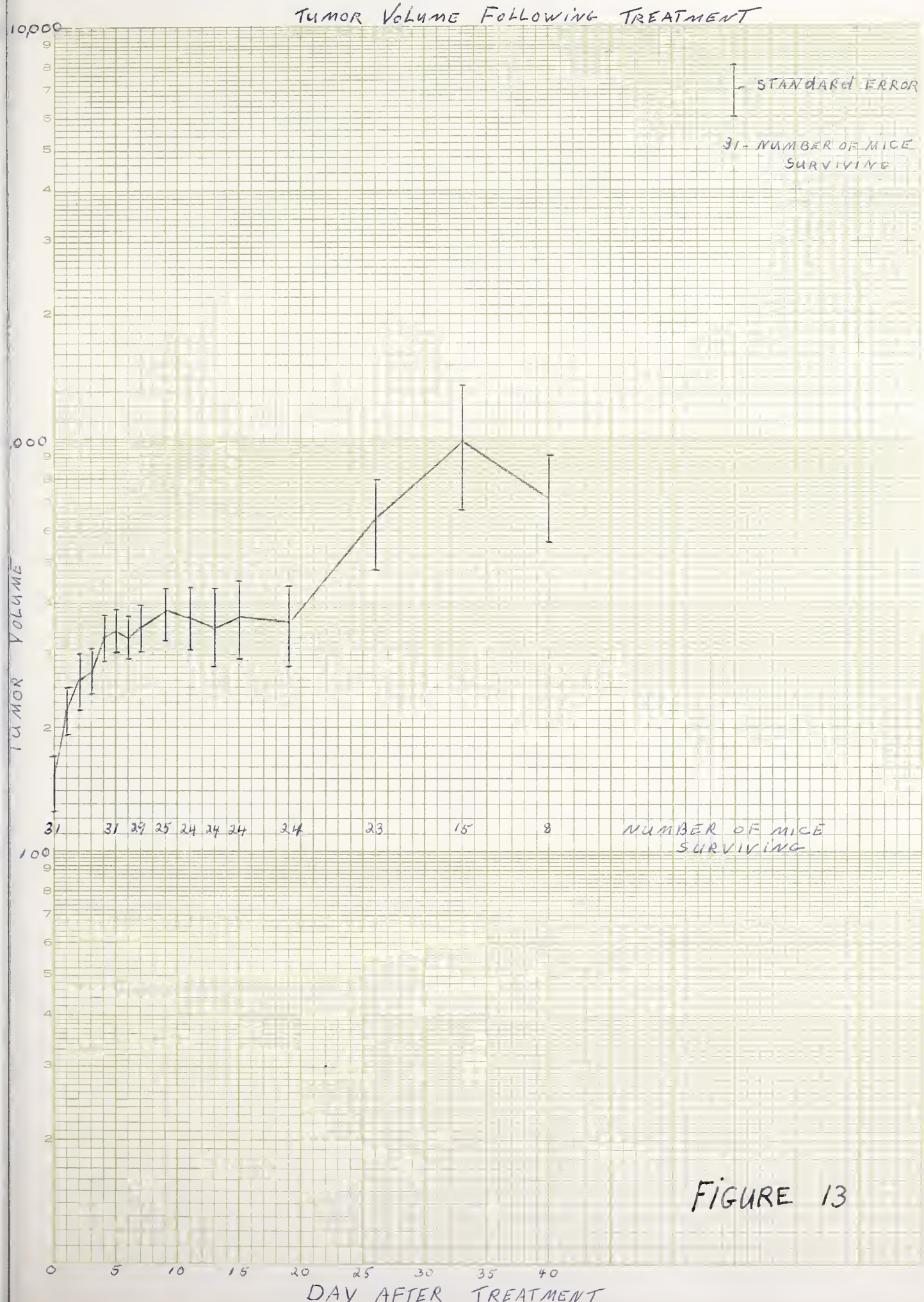


FIGURE 13

FIGURE 14 - GROUP VI X-RAY 24 HRS. BEFORE 5FU

TUMOR VOLUME FOLLOWING TREATMENT

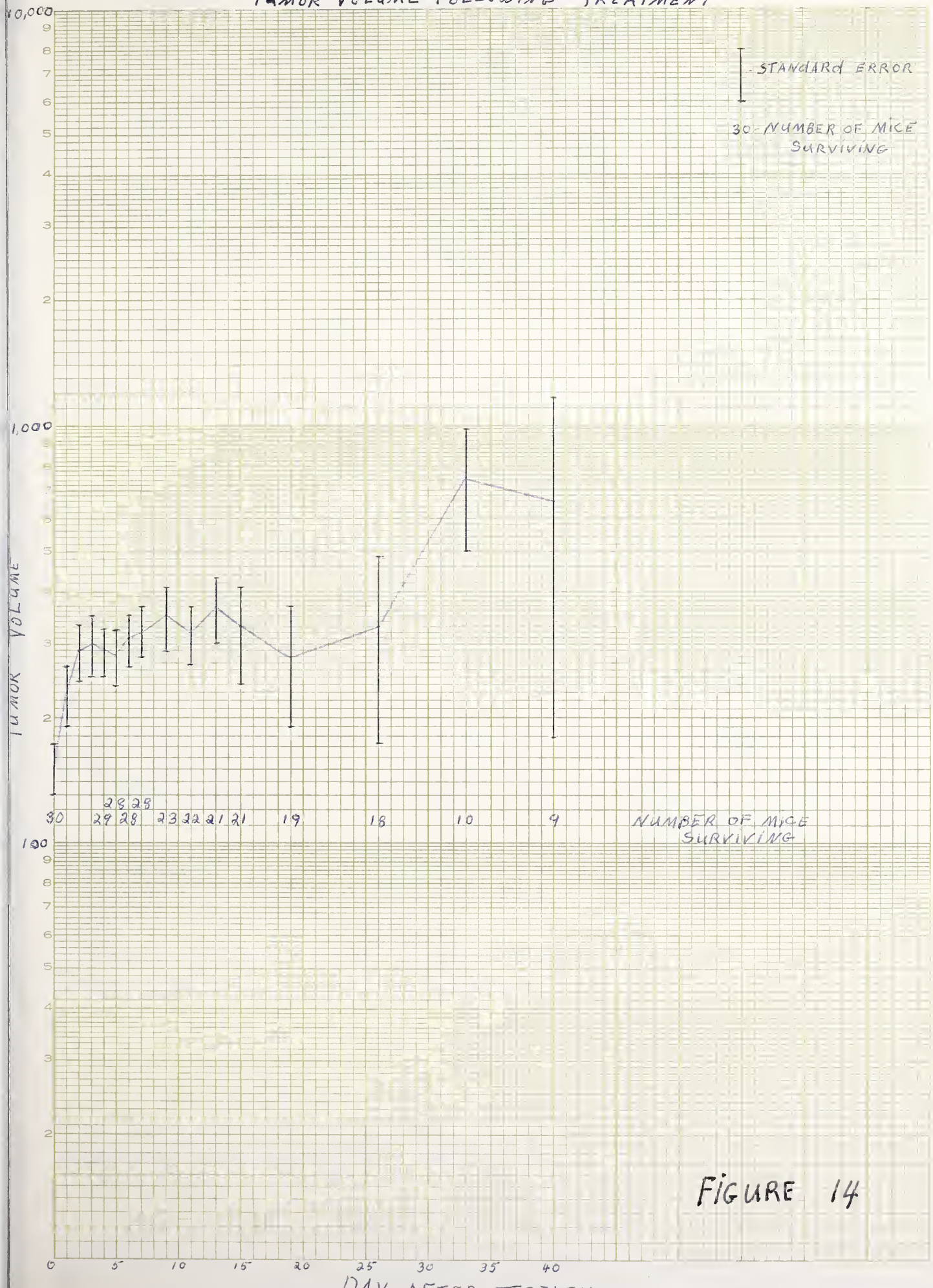


FIGURE 14

FIGURE 15 - GROUP VII - CONTROL

TUMOR VOLUME FOLLOWING TREATMENT

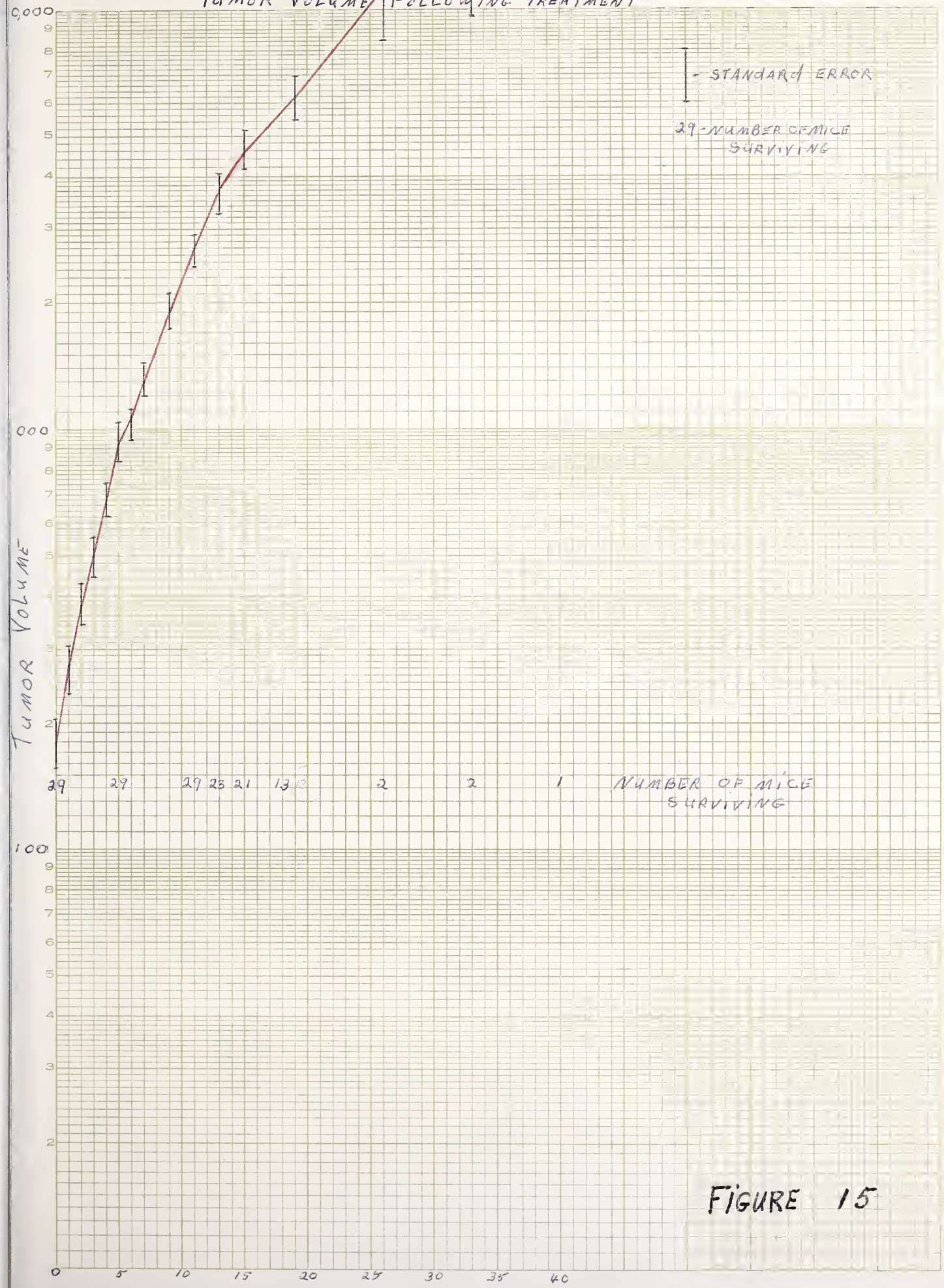


FIGURE 15

FIGURE 15 - GROUP VII - CONTROL

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